FURTHER STUDIES ON THE GEOTROPISM OF PARAMECIUM CAUDATUM.[1]

SAKYO KANDA,

THE PHYSIOLOGICAL INSTITUTE OF THE KYUSHA IMPERIAL UNIVERSITY, FUKUOKA, JAPAN.

(With two figures.)

CONTENTS.

I. INTRODUCTORY.

As evidence of the existence of proper mechanical conditions in protoplasm as a basis for the "statocyst theory" of geotropism in *Paramecium*, Lyon states: "The animals were strongly centrifuged for several minutes in the hæmatocrit attachment. Microscopic examination showed that certian dark granules originally distributed were now aggregated in one end, usually the anterior. It is thus seen that differences in specific gravity exist in the protoplasm of this animal."[2] In previous experiments in which he attempted to test Lyon's results, the writer "could obtain nothing definite"; and the tentative suggestion was made that "immediately after centrifuging a capillary tube containing *Paramecia*, and in which the latter can not turn around, we may stain them with some dyes and determine the effects

[1] From the Physiology Laboratory of the University of Minnesota.

[2] Lyon, E. P., 1905, "On the Theory of Geotropism in *Paramecium*, *Am. Jour. Physiol.*, Vol. 14, p. 430.

of the centrifugal force upon the protoplasmic materials of the organisms."[1]

While the writer was conducting experiments with a vital stain method in the physiological laboratory of the University of Minnesota, and after he had obtained some valuable results, he learned that McClendon had already published satisfactory results in 1909.[2] The writer found that McClendon's method showed better results than those obtained with vital stains and has, therefore, adopted that method for this investigation, although not able to agree with all of McClendon's interpretations.

This paper attempts to show that *Paramecium caudatum* contains protoplasmic substances of different specific gravities, and subjects to further experimental examination the various phenomena of geotropism in the animal, described by various investigators. The experimental work was done during the academic year of 1913–1914, while the writer was holding a Shevlin Fellowship in the medical school of the University of Minnesota.

II. Material.

A dense culture raised in hay infusion from a single individual was used.

III. Experimental.

1. *The Specific Gravity of Paramecium caudatum.*

As to the difference of the specific gravity of the *Paramecium* between Lyon's 1.048 or 1.049 and the writer's 1.037 or 1.037 $\pm$ 0.003, the latter suggested[3] a possible source of error in Lyon's experiments. The writer this time examined the subject more carefully.

He prepared a gum-arabic solution using distilled water, as in the case of his previous experiments. He dialyzed the neutral solution through parchment paper. The specific gravity of the solution so prepared was determined by means of a U-shaped picnometer, as 1.0426.

[1] Kanda, Sakyo, 1914, "On the Geotropism of *Paramecium* and *Spirostonum*," Biol. Bull., Vol. 26, p. 22.

[2] McClendon, J. F., 1909, "Protozoan Studies," *Jour. Ex. Zool.*, Vol. 6.

[3] Kanda, Sakyo, *loc. cit.*

Then the writer made many small tubes for hæmatocrit attachment, of about 4.7 cm. long and 0.3 cm. in diameter, inside measure. One end of each tube was sealed. One of these tubes was filled with the gum-arabic solution mentioned above and the number of drops which were put in the tube was counted. Now, to the number of the drops of the solution, one drop of water was added. Thus the difference of the specific gravity of the solution before and after the addition of one drop of the latter to the number of drops of the former could be estimated. The writer thus found that the addition of only one small drop of water to the gum-arabic solution of the known specific gravity, that is, 1.0426, lowered the latter to 1.0415. And when another drop of water was added to this, it became about 1.0404. The addition of one drop of water, therefore, lowered the specific gravity of gum-arabic solution about 0.0011.

A pair of tubes was thus prepared with a definite number of drops of the gum-arabic solution of the specific gravity 1.0415. This pair was centrifuged as usual. Then on the top of the solution of one tube, one drop, and on the other, two drops of water containing dense *Paramecia* were added. The tubes were again centrifuged for two minutes with a speed of about 7,300 revolutions per minute. The results were determined by means of a magnifying glass immediately after the centrifugalization. All the procedure was the same as the writer described in his previous paper. Thus the nearest possible density of the animal was obtained:

TABLE I.

I.	Tube 1. Dens. of gum-arab. sol. 1.0404. All stay at upper part.
	Tube 2. Dens. of gum-arab. sol. 1.0393. Majority stay near middle.
II.	Tube 1. Dens. of gum-arab. sol. 1.0382. Many stay at middle.
	Tube 2. Dens. of gum-arab. sol. 1.0371. A few go to bottom.

This was carefully tried with different cultures and no practical difference was found. The writer, therefore, concludes that the specific gravity of *Paramecium caudatum* is between 1.0382 and 1.0393.

In comparison with the writer's previous results, that is, 1.037 or 1.037 ± 0.003, the difference is not great. In his previous experiments, however, he was not critical enough to test the lowering of the specific gravity of the gum-arabic solution after one or two drops of water containing *Paramecia* were added to the tubes. The writer, therefore, considers that the present result is more correct than the previous one.

A more accurate estimation could possibly be made by having the animals before adding them to the centrifuge tube in gum solution of a density only a little less than that of the animals. The lowering of specific gravity by mixture would thus be lessened. Nevertheless, the writer believes that the assumption of 1.039 as the specific gravity of *Paramecium caudatum* is close enough for biological purposes.

2. *The Effects of Centrifugal Force on the Protoplasm of Paramecium caudatum.*

The specific gravity of the animal being known as about 1.039, a gum-arabic solution of higher specific gravity, that is, about 1.1, was prepared. If *Paramecia* are strongly centrifuged in such a solution, they all should be suspended in the solution. And the heavier end of the animals should, also, be passively thrown away from the axis of the centrifuge. Now the writer put a certain number of drops of the gum-arabic solution in a pair of hæmatocrit tubes already described, and on the top of each, one drop of water containing dense *Paramecia* was added. The solution and the water in the tubes were well mixed by means of a fine glass needle. The tubes were centrifuged for fifteen minutes at a velocity of 108.3 revolutions per second and with radius of 2.5 cm. to 7 cm.

The paramecia with some of the gum-arabic solution, after being centrifuged, were sucked out in a capillary tube and were killed in 1 per cent. chromic acid, being left in the solution for about one minute. Then they were stained by McClendon's method, "in Biondi's methyl green, orange G and acid fuchsin mixture with a little less fuchsin and of about one fourth saturated strength"[1] for about four minutes, dehydrated and mounted.

[1] McClendon, J. F., *loc. cit.*

The microscopic examination of the preparations showed that the darker and heavier substances and crystal-like materials lay in the extreme anterior end of the animal which was thrown away from the axis of the centrifuge. Next to these came the micro-

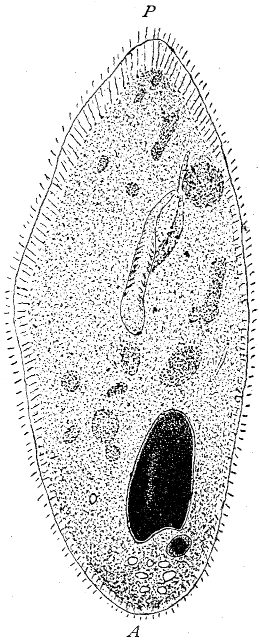

FIG. 1.

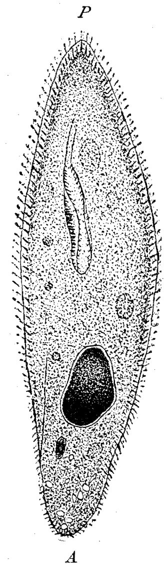

FIG. 2.

FIGS. 1 and 2. Camera drawings of *Paramecium caudatum*. *A*, anterior end; *P*, posterior end.

nucleus and then the macro-nucleus. The chromatin which was stained green, seemed to have been precipitating to the outer nuclear wall, since this part of the nucleus was stained much darker than the inner, as Figs. 1 and 2 have shown. The "plasmosomes" and cell granules were stained orange, but the cilia did not show very distinctly. The writer thus found in confirmation of Lyon's and McClendon's results that *Paramecium* contains protoplasmic materials of differing specific gravities. Figs. 1 and 2 show specimens drawn with the aid of a camera attachment.

That the anterior end of the animal, when centrifuged, was thrown away from the axis of the centrifuge presumably on ac-

count of its heavier weight, was originally demonstrated by Lyon. This was the strongest in his experiments to cast aside the "mechanical theory." The writer supported him by furnishing results obtained by improved methods to meet Harper's criticism.[1] Harper insisted that in strong centrifugalization, "the same effect is produced at the outset as by mechanical agitation, *i. e.*, the reaction changes to positive." In other words Harper claims that the head is turned outward by a positive response on the part of the organism, and not passively, as claimed by Lyon. The writer showed in his previous paper that Harper could not be right.

However, McClendon thinks that "the geotropic reaction" of the animal "may be strong enough to turn the anterior end in the opposite direction" toward the axis of the centrifuge against the centrifugal force. This needs a serious examination. If the anterior end of the animal is considered "heavier" than the posterior, as Lyon and Kanda hold, it is hardly conceivable that such a small animal as *Paramecium* could orient itself with the heavier end toward the axis of the centrifuge against the force McClendon used. On the other hand, if the posterior end is regarded "heavier" than the anterior, as Verworn[2] and Harper hold, one meets the same difficulty to conceive how the animal can orient itself with the "lighter" anterior end away from the axis of the centrifuge against the much greater force used by Lyon and the writer. McClendon admits that "this is usually the initial orientation," but seems to think the animals may occasionally turn around later by an active reaction. The writer must confess that he cannot conceive its possibility. He found one individual among several dozen examined which was thrown with the posterior end away from the axis of the centrifuge. He considers, however, that this was a mere accident, the animal being oriented in that direction at the beginning of centrifugalization and forced into the capillary before passive turning could occur. McClendon's case was also probably exceptional and moreover had been centrifuged twenty-four hours, during which

[1] Harper, E. H., 1911, "The Geotropism of *Paramecium*," *Jour. Morph.*, Vol. 22, p. 998.

[2] Verworn, Max, 1889, "Psycho-Physiol. Protisten-Studien," Jena: Gustav Fischer, p. 121.

admitted changes in specific gravity had occurred, due to loss of water. If this loss were chiefly from the posterior part, the changed orientation would be explained as a passive instead of an active process. The writer, therefore, holds still that these animals, when strongly centrifuged, are *passively* thrown with their anterior ends away from the axis of the centrifuge. Therefore, the anterior end is heavier. The negatively geotropic orientation in the normal animals is, therefore, an active process and the mechanical theory is not correct.

McClendon "found the time elapsing before the return of the negatively geotropic reaction to roughly correspond to the time required for the return of the nuclei to their normal position." The writer also found that this was the case. According to McClendon, "this might indicate that nuclei in normal position acted as statoliths." It is hard to decide for or against this for at the time of "the return of the nuclei to their normal position," other heavy substances, "phosphate crystals," for instance, which were precipitated in the extreme anterior end of the animal seem, also, to distribute in their original position. In other words, about at the time of return of the nuclei to their normal position, all the protoplasmic substances of the animal, which were disturbed by the centrifugal force from their original distribution, recover from the disturbance. The result is that the animal resumes the negatively geotropic reaction. Such consideration may suggest that the whole organism in normal conditions acts as a "statecyst," and all the heavy substances as "statoliths," not merely the nuclei. Here lies the significance of Lyon's conception that "for internal stimulation the relation of the parts of the cell to each other must be changed in some way by gravity. Stresses or pulls which occur when the organism is in one position with respect to the vertical, must be changed in another position." For the present we cannot decide in favor of any particular organ or constituent of the cell as the basis of the reaction.

3. Does temperature reverse the negative geotropism of *Paramecium caudatum?*

From his observations on the effects of raising and lowering temperature on the geotropism of this animal, Sosnowski con-

cludes: "Es hat sich dabei herausgestellt, dass durch Einwirkung höher Temperaturen . . . bei vielen Culturen vorübergehend positiver Geotropismus hervorgerufen werden kann." And "durch die Temperaturerniederingung (bis + 2°) konnte ich auch bei den sehr dazu geeigneten Aquarien keinen positiven Geotropismus hervorufen."[1] On the contrary, Moore[2] states: "Several tubes were left for three hours in a thermostat at a temperature of 26°–28° C. The *Paramecia* collected at the top in dense clusters." And "tube *B* was placed in a larger tube filled with water and surrounded by a mixture of ice and salt, the temperature being kept as nearly as possible at 1° C. In ten minutes the *Paramecia* in tube *B* were massed at the bottom, and two hours later were still massed there."

Sosnowski and Moore, however, gave no consideration to the phenomena of the convection currents which would invariably occur, when water was cooled or heated. That water currents would produce effects on the geotropism of the animal was mentioned by the writer in his previous paper. The animals are rheotropic. Moreover, the animals being so small cannot resist any strong current. Consequently they would be passively carried by the current, when it was strong. There was, therefore, needed a special device to minimize these effects as much as possible.

Dewar vacuum tubes served to some extent for this purpose. The tubes were 3.2 cm. in diameter and 27 cm. long inside measure. Three tubes, one for control and the others for experiments, cold and warm, were used for one series of experiments. It was noted that warm water of 30° or 35° C., which was placed in one of the tubes, with a rubber stopper, cooled off about 2° C. after one hour, and water of 1° to 4° C. in the other became warmer 2° C. in about half an hour. Temperature of the control, that was room temperature, was about 20° C. and fairly constant. The dense culture of animals that were thoroughly washed was cooled or warmed as desired. And a few drops of the culture so

[1] Sosnowski, J., 1899, "Untersuchungen über die Veränderungen der Geotropismus bei *Paramecium aurelia*," *Bull. Intern. d. l'Acad. d. Sci. d. Cracovie*, S. 134.

[2] Moore, Anne, 1903, "Some Facts concerning the Geotropic Gathering of *Paramecium*," *Am. Jour. Physiol.*, Vol. 9, pp. 239 and 240.

prepared were transferred by means of a pipette into the Dewar tube with special care to make the least current. The tube was closed with a rubber stopper. The results based on many experiments are given in Table I.

TABLE I.

EFFECTS OF TEMPERATURE (?).

Time.	Cold.	Control.	Warm.
	1°–2° C	20° C.	20°–30° C.
Immediately after.	Very sluggish and hardly active.	Majority swim downward.	Abnormally active and majority swim down.
5–10 m. later.....	Not very active and scattered all over.	Many at the lower part.	Many at the lower part.
20–30 m. later....	A little more at the lower part and becoming somewhat active. The temp. about 4° C.	Many at the upper part.	A little more at the lower part. The temp. about 29° C.

Cooling water to 3°, 4°, or 5° C. and warming to 35° C. were also tried. At a temperature 5° C., the animals were somewhat active, and showed a tendency to swim downward. Another series of experiments was also carried out in an incubator and in an ice box. Each experiment lasted three days. The temperature in the incubator ranged from 30° C. to 34° C. In one series of the experiments, the animals so treated in the incubator were "positively geotropic"? That is to say, a majority of the animals always staid at the bottom of the tube, as Sosnowski observed. But in others, the results were very irregular and variable. Experiments in the ice box were not satisfactory, because it was very hard to keep the temperature constantly low.

The writer found that the animals always swam downward, whenever transferred, no matter how carefully it was done. This occurred invariably even in water of the same temperature as the culture, when the animals were transferred into it. It seems to the writer, therefore, to be possible that this reaction was mistaken as reversibility of geotropism of the animals by temperature. That the animals swim downward, *i. e.*, their negative geotropism is reversed, whenever transferred, would suggest

that mechanical agitation of transferring may be responsible for it. Sosnowski also suggested this factor in one case, "die Temperaturerhöhung und Erschütterung sich zu summieren scheinen" The reversibility of the negative geotropism of the animals by mechanical "shock" or agitation is a well-known. fact. The water current may also help it to some extent.

From these results the writer concludes that so-called reversibility of the negative geotropism of *Paramecium* by temperature is extremely doubtful. Especially in such cold water as near 1° C., the animal is almost, if not thoroughly anesthetized. In consequence, it sinks by its weight, though Moore thinks it is "reversed."

4. Do chemicals reverse the negative geotropism of *Paramecium caudatum?*

According to Sosnowski, "durch Zusatz geringerer Mengen von Säuren oder Alkalien (1 bis 3 c.c. Salzsäure oder Natronlauge von 0.5 per cent. auf 20 c.c. Culturflüssigkeit) kann man verubergehenden positiven Geotropismus hervorufen." Moore also states that "in *n*/16 NaCl, they went to the bottom almost immediately, but shortly after died." In a solution of calcium chloride isosmotic with *n*/32, "they went immediately to the bottom."

The writer attempted to test these statements of Sosnowski and Moore. First of all, he thoroughly washed the animals in boiled then cooled tap water. Chemical solutions from the strongest to the weakest were carefully prepared using boiled tap water. A few drops of culture containing dense *Paramecia* were transferred with special care to avoid making water currents as much as possible. The results given in Table II. are to be considered as the best parts of many series of experiments that were quite extensively carried out.

As the table shows, the majority of the animals always swam downward, *i. e.*, were positively geotropic, whenever transferred into any solution as well as in the control, *i. e.*, transfer into culture water. Among the substances used, perhaps, $CaCl_2$ solution "affected" the animals most. In this respect, Moore's observation seems to be confirmed, but it needs further consideration. In any solution, the animals that swam to the bottom did

not stay there very long. They resumed their negative geotropism within one hour. Sosnowski already observed the same and stated that "wenn sich darauf die Tiere wieder an der Oberfläche sammeln, kann man durch Zusatz einer neunen Quantitat Säure resp Alkali wieder die Ansammlung am Boden versanlassen." This statement is true. But it should be remembered that the same is also true when some tap water is added. It seems to the

Table II.

Effects of Chemicals.

	Room Temp. 20° C. Time.					
	Immediately after Treatment.	5 Minutes after Treatment.	10 Minutes after Treatment.	20 Minutes after Treatment.	30 Minutes after Treatment.	60 Minutes after Treatment.
Control	90% or more swim downward.	About 85% at bottom.	Number at bottom decreasing.	Scattered all over.	About 70% at upper part.	About 80% at top.
0.01 m. or 0.0133 m. $CaCl_2$	Abnormally active and 95% or more swim downward.	About 95% at bottom.	About 80% at bottom.	About 60% at lower part.	60% or more at upper part.	About 85% at top.
0.01 m. or 0.0133 m. Na_2CO_3 or $NaHCO_3$	About 95 % swim downward.	85% or more at bottom.	Scattered all over.	About 70% near top.	80% or more at top.	About same.
.05% HCl[1]	90% or more swim downward.	70% or more at bottom.	Scattered all over.	About 60% at upper part.	About 70% near top.	About same.

writer that this means that the so-called reversible effect of chemicals on the negative geotropism of *Paramecium* is not specific but general. Moreover, the animals, whenever transferred to another receptacle without changing the solution, swim downward, and reversed animals do not stay long at the bottom.

There is, therefore, reason to believe that this is the same phenomenon that we met in the temperature experiments. The writer concludes, therefore, that mechanical "shock" or agitation of transferring, or chemical "shock," is chiefly responsible for so-called reversible effect of chemicals on the negative geotropism of *Paramecium* but not chemicals themselves.

Besides the chemicals mentioned above, sodium chloride,

[1] This was adopted as approximately Sosnowski's strength.

magnesium chloride, cane sugar, ethyl alcohol and chloroform in appropriate strength were tried but no better results were obtained.

IV. Summary and Conclusions.

1. The specific gravity of living *Paramecium caudatum* is between 1.0382 and 1.0393.

2. *Paramecium caudatum* contains protoplasmic materials of different gravities.

3. *Paramecium caudatum*, when strongly centrifuged, assumes a position with its anterior end directed away from the axis of the centrifuge. In so doing, it must be passively thrown by the centrifugal force, because the centrifugal force used was altogether too strong to be resisted by the animal. For this reason, its anterior end must be heavier than its posterior end. The negatively geotropic orientation, therefore, is an active process and the mechanical theory is not correct.

4. The whole organism seems to be a "statocyst," since the recovery of negative geotropism after centrifugalizing is synchronous with the reëstablishment of normal relations of substances in the cell.

5. So-called reversibility of the negative geotropism of *Paramecium caudatum* by temperature and by chemicals is extremely doubtful. Mechanical "shock" or agitation is chiefly responsible for the reversal one sees in such cases.

In conclusion the writer wishes to acknowledge his indebtedness to Professor E. P. Lyon, under whose direction this work was completed, and who gave valuable suggestions and criticism of the work and manuscript. His thanks are due also to Dr. J. F. McClendon, who gave his generous help and suggestions during the experiments.